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(54) Title: RESPIRABLE DRIED POWDER FORMULATION COMPRISING DRUG LOADED NANOPARTICLES

(57) Abstract: A pharmaceutical formulation for administration by aerosol inhalation to the lung and comprising spray-freeze dried powder comprising nanoparticles loaded with at least one active principle is described herein. The powder provides for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region.

TITLE OF THE INVENTION

RESPIRABLE DRIED POWDER FORMULATION COMPRISING DRUG LOADED NANOPARTICLES

This application claims the benefit of provisional Application No. 60/689,099 filed June 10, 2005. The contents of this application are incorporated into this specification by reference.

FIELD OF THE INVENTION

[0001] The present invention broadly relates to a drug delivery system. More particularly, the present invention relates to a respirable dried powder formulation comprising drug loaded nanoparticles.

BACKGROUND OF THE INVENTION

[0002] Cancer is a prevalent and devastating disease that affects many people each year. It is one of the leading causes of death worldwide. Indeed, the lifetime probability of developing cancer is 41% for males and 38% for females, of which about 20% will contract primary carcinoma of the lung - the leading cause of cancer mortality (Statistics Canada, 2003).

[0003] Of all the cancers, lung cancer has the highest mortality rate. Despite the availability of many cancer drugs it has been difficult, almost impossible, to improve cure rates or survival rates for patients suffering from lung cancer. Conventional treatments of primary and metastatic lung cancers are only marginally successful with a five-year survival rate of 14% [Placke, M.E., Zimlich, W.C., Ding, J.D., Westaway, D.J., Imondi, A.R. 2002; *Targeted aerosol therapy for the treatment of lung cancer*. Respiratory Drug Delivery. VIII: 15 – 23]. One of the main reasons for this lack of success is the present inability to deliver adequate amounts of drug(s) (*i.e.* efficacious concentrations of anticancer agent) to the tumor cite without causing debilitating and life-threatening toxicities in the patient. Indeed, most chemotherapeutic agents used to treat cancer are highly toxic to both normal and tumor tissues.

[0004] Treatment for non-small cell lung cancer (NSCLC) commonly is surgical resection with the possibility of adjuvant chemotherapy and/or radiation therapy. Radiation and combination chemotherapy is typically utilized for small-cell lung cancer (SLC). Complete surgical resection of N1 NSCLC carries a 50% five-year survival rate, while combination chemotherapy has a one-year survival rate of 40%. Some causes of ineffectual chemotherapy for NSCLC include tumor resistance to drug penetration and irregular tumor vasculature [Jain, R., 1994; *Barriers to drug delivery in solid tumors.* Scientific American; 58 – 65], resulting in an insufficient concentration of active agent at cancer cell locations.

[0005] In order to increase the dosages at the tumor cite, some attempts have been made to deliver the anticancer agents directly to the tumor location or to the region of the tumor. For lung cancer, this can be achieved through the inhalational drug delivery route, which also reduces drug dosage and systemic toxicity. However, it is well known that a large percentage of aerosolized drug intended for the lung is swallowed.

[0006] Tatsamura *et al.* [Jap. J. Cancer Cln., 1983, Vol. 29, pp. 765-770] reported on the feasibility of drug inhalation therapy. It was reported that fluorouracil (5-FU) was effective for the treatment of lung cancer in a small group of human patients when administered directly to the lung by aerosolization. They referred to this as nebulization chemotherapy.

[0007] Desai *et al.* [U.S. Patent 5,439,686] teach compositions where a pharmaceutically active agent is enclosed within a polymeric shell for administration to a patient. One of the routes of administration listed as possible for the compositions is by inhalation. However, no tests using the inhalational route of administration appear to have been made.

[0008] Nanoparticle (NP) drug formulations and delivery through oral, intravenous, and inhalation routes, have received considerable attention. It was reported that the interstitial pressure in tumors larger than 5 mm in diameter was uniform and larger than the pressure surrounding the tumor [Jain, R., 1994; *Barriers to drug delivery in solid tumors.* Scientific American; 58 – 65]. Consequently, drug

penetration into a tumor is largely a diffusive process, and smaller particles have an increased probability of entering, and exiting, a tumor. However, the main drawback with nanoparticle drug preparations is the difficulty in achieving high loads of drug [Zhang, Z., Liao, G., Nagai, T., Hou, S. 1996; *Mitoxantrone polybutyl cyanoacrylate nanoparticles as an anti-neoplastic targeting drug delivery system.* Int. J Pharm. 139: 1-8]. Notwithstanding the previously cited drawback, the nanoparticle drug configuration does offer some generalized benefits such as improved drug targeting capability, decreased toxicity, and increased formulation stability.

[0009] The concept of "*Trojan nanoparticles*" or "*cluster bombs*" [Tsapis, N., Bennett, D., Jackson, B., Weitz, D.A., Edwards, D.A. 2002; *Trojan particles: large porous carriers of nanoparticles for drug delivery.* PNAS. 99: 12001-12005] was developed because lipid nanoparticles sometimes decrease the toxicity of a therapeutic agent while synthetic nanoparticles such as polycyanoacrylate nanoparticles have been observed to reverse multi-drug resistance (MDR) in cancer cells [Hu, Y.P., Jarillon, S., Dubernet, C., Couvreur, P., Robert, J. 1996; *On the mechanism of action of doxorubicin encapsulation in nanospheres for the reversal of multidrug resistance.* Cancer Chemotherapy & Pharmacology. 37: 556-560. DeVerdiere, A.C., Dubernet, C., Nemati, F., Soma, E., Appel, M., Bernard, S., Puisieux, F., Couvreur, P. 1997; *Reversion of multidrug resistance with polyalkylcyanoacrylate nanoparticles: towards a mechanism of action.* British J. of Cancer. 76: 198-205]. A lyophilized doxorubicin powder for intravenous use upon aqueous re-suspension has been produced [DeVerdiere, A.C., Dubernet, C., Nemati, F., Soma, E., Appel, M., Bernard, S., Puisieux, F., Couvreur, P. 1997; *Reversion of multidrug resistance with polyalkylcyanoacrylate nanoparticles: towards a mechanism of action.* British J. of Cancer. 76: 198-205]. Resmycin™, an inhalable doxorubicin containing solution, is currently undergoing Phase II clinical trials for the treatment of lung tumors.

[0010] Liposomes have been extensively examined as a drug delivery system for various reasons. Liposomal encapsulation can significantly increase the solubility of therapeutic agents that are otherwise poorly soluble [Mohammed, A. R., Weston, N., A.G.A Coombes, Fitzgerald, M., Perrie, Y. 2004; *Liposome formulation of poorly water soluble drugs: optimization of drug loading and ESEM analysis of stability.* Intl. J. Pharm. 285: 23 – 34] and may also increase efficacy [Wong, J. P.,

Yang, H., Blasetti, K. L., Schnell, G., Conley, J., Schofield, L. N. 2003; *Liposome delivery of ciprofloxacin against intracellular Francisella tularensis infection.* J. Control Release. 92(3): 265 – 273]. Moreover, liposomal encapsulation may further reduce the toxicity of the therapeutic agent. Evidence suggests that the cardiotoxicity of doxorubicin is reduced through liposomal encapsulation [Working, P. K., Newman, M. S., Sullivan, T., Yarrington, J. 1999; *Reduction of the cardiotoxicity of doxorubicin in rabbits and dogs by encapsulation in long-circulating, pegylated liposomes.* J. Pharmacol. Exp. Ther. 289: 1128 – 1133].

[0011] There thus remains a need for an improved drug delivery vehicle for delivering a therapeutically efficacious concentration of an antineoplastic drug to the tumor cite or to region of the tumor. More specifically, there remains a need for an improved drug delivery vehicle for inhaled aerosol delivery to the lungs. There also remains a need for a method for the preparation of such an inhalable drug delivery vehicle.

[0012] The present invention seeks to meet these and other needs.

[0013] The present invention refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0014] The present invention relates to a dry powder drug delivery vehicle suitable for inhalation aerosol therapy. More specifically, the present invention relates to a dry powder drug delivery vehicle comprising an active principle or diagnostic, suitable for inhalation aerosol therapy. Yet more specifically, the present invention relates to a dry powder drug delivery vehicle comprising an active principle or diagnostic, suitable for inhalation aerosol therapy delivering the active principle or diagnostic to the respiratory tract or lungs of an individual. In an embodiment, the active principle or diagnostic may be selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers of various types, imaging enhancers, and combinations thereof. In a particular embodiment, the active principle is an antineoplastic agent for the treatment of lung cancer. In yet

a more particular embodiment, the present invention relates to a dry powder drug delivery vehicle obtained by spray-freeze drying and comprising a carrier and an active principle or diagnostic which is suitable for inhalation aerosol therapy.

[0015] In an embodiment, the present invention relates to a dry powder drug delivery vehicle suitable for inhalation aerosol therapy comprising a carrier, a nanoparticle-forming element and an active principle or diagnostic. In an embodiment of the present invention, the nanoparticle-forming element may be lipid based. In a further embodiment of the present invention, the nanoparticle-forming element may by a monomer capable of generating a polymeric matrix. In yet a further embodiment, the nanoparticle-forming element is a cyanoacrylate. In a particular embodiment, the active principle is an antineoplastic agent for the treatment of lung cancer. For aerosol delivery to the lung, the carrier material must be non-toxic and capable of releasing the nanoparticles at the target site, such as by dissolving in the aqueous environment of the epithelium.

[0016] In an embodiment, the present invention relates to a pharmaceutical composition comprising a carrier, a nanoparticle-forming element and an active principle or diagnostic. In a more particular embodiment, the pharmaceutical composition is suitable for inhalation aerosol therapy. In yet a more particular embodiment, the pharmaceutical composition is suitable for inhalation aerosol therapy whereby the active principle or diagnostic is delivered to the respiratory tract or lungs of an individual.

[0017] In an embodiment, the present invention relates to a pharmaceutical formulation comprising spray-freeze dried powder, the powder comprising nanoparticles loaded with at least one active principle, the powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region, the formulation being administered by aerosol inhalation to the lung.

[0018] In an embodiment, the present invention relates to a drug delivery vehicle for inhalation aerosol therapy comprising spray-freeze dried powder,

the powder comprising nanoparticles loaded with at least one active principle, the powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region.

[0019] In an embodiment, the present invention relates to a method of manufacturing a dry powder drug delivery vehicle suitable for inhalation aerosol therapy. In a more particular embodiment, the present invention relates to a method of manufacturing a dry powder drug delivery vehicle suitable for inhalation aerosol therapy comprising an active principle or diagnostic as previously described herein. In yet a more particular embodiment, the present invention relates to a method of manufacturing a dry powder drug delivery vehicle suitable for inhalation aerosol therapy comprising an active principle or diagnostic as previously described herein, whereby the active principle or diagnostic is delivered to the respiratory tract or lungs of an individual.

[0020] In an embodiment, the present invention relates to a method of formulating a powder containing nanoparticles for inhalation aerosol delivery to the lung, the method comprising the steps of i) mixing a cyanoacrylate monomer with a liquid carrier and at least one active principle to create a suspension comprising nanoparticles loaded with the at least one active principle; and ii) submitting the suspension to spray freeze-drying producing carrier particles suitable for aerosol delivery to the lung. The so-obtained carrier particles provide for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the carrier particles in the airway surface liquid following deposition of the carrier particles throughout the tracheobronchial region, the carrier particles being administered by aerosol inhalation to the lung.

[0021] In an embodiment, the present invention relates to a method of treating lung cancer in a patient comprising the step of administering to the patient a formulation comprising spray-freeze dried powder, the powder comprising nanoparticles loaded with at least one active principle, the powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region, the formulation being administered by

aerosol inhalation to the lung.

[0022] In an embodiment, the present invention relates to a use of the dry powder drug delivery vehicle as described herein for delivering an active principle or diagnostic to the respiratory tract or lungs of an individual. The active principle or diagnostic may be selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers of various types, imaging enhancers, and combinations thereof. In a particular embodiment, the active principle is an antineoplastic agent for the treatment of lung cancer.

[0023] In a particular embodiment, the present invention relates to the use of a dry powder drug delivery vehicle comprising an antineoplastic agent as described herein for treating cancer of the respiratory tract or lung.

[0024] In a particular embodiment, the present invention relates to a dry powder drug delivery vehicle suitable for inhalation aerosol therapy comprising a lactose or dextran carrier matrix further comprising lipid or cyanoacrylate-based nanoparticles, the nanoparticles comprising an active principle or diagnostic selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers of various types, imaging enhancers, and combinations thereof. In a more particular embodiment, the active principle or diagnostic is an antineoplastic agent. In yet a more particular embodiment, the antineoplastic agent is an anthracycline such as doxorubicin or its corresponding salt (*i.e.* doxorubicin hydrochloride).

[0025] In an embodiment, the present invention relates to formulations suitable for inhalation aerosol therapy, the formulations comprising a dry powder drug delivery vehicle comprising a carrier, a nanoparticle-forming element and an active principle or diagnostic. In a particular embodiment, the formulations comprise a drug delivery vehicle comprising a carrier and liposomal nanoparticles loaded with an anthracycline such as doxorubicin or its corresponding salt (*i.e.* doxorubicin hydrochloride). In yet a further particular embodiment, the formulations comprise a drug delivery vehicle comprising a carrier and cyanoacrylate-based nanoparticles loaded with an anthracycline such as doxorubicin or its corresponding salt (*i.e.* doxorubicin hydrochloride).

[0026] In an embodiment of the present invention, the dry powder drug delivery vehicle as described herein may be obtained by spray-freeze drying.

[0027] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indication specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

[0029] **Fig 1**: **Fig. 1** shows a comparison of the butylcyanoacrylate nanoparticle size distributions of particles loaded with doxorubicin or without doxorubicin loading;

[0030] **Fig 2**: **Fig. 2** shows a cytotoxicity comparison of plain doxorubicin (Dox), unloaded cyanoacrylate nanoparticles (Naked-NPD), and doxorubicin-loaded cyanoacrylate nanoparticles (NPD-13 & NPD-14) on cell line H460 (the error bars represent the mean standard error on 3 measurements);

[0031] **Fig 3**: **Fig. 3** shows a cytotoxicity comparison of plain doxorubicin (Dox), unloaded cyanoacrylate nanoparticles (Naked-NPD), and doxorubicin-loaded cyanoacrylate nanoparticles (NPD-13 & NPD-14) on cell line A549 (the error bars represent the mean standard error on 3 measurements);

[0032] **Fig 4**: **Fig. 4** shows a cytotoxicity comparison of plain doxorubicin (Dox), unloaded cyanoacrylate nanoparticles (Naked-NPD), and doxorubicin-loaded cyanoacrylate nanoparticles (NPD-13 & NPD-14) on cell line DU145 (the error bars represent the mean standard error on 3 measurements);

[0033] **Fig 5**: **Fig. 5** shows a cytotoxicity comparison of plain doxorubicin (Dox), unloaded cyanoacrylate nanoparticles (Naked-NPD), and doxorubicin-loaded cyanoacrylate nanoparticles (NPD-13 & NPD-14) on cell line SK-NSH (the error bars represent the mean standard error on 3 measurements);

[0034] **Fig 6**: **Fig. 6** shows Lagrangian simulation results displaying the predicted regional deposition of doxorubicin (generation 0 represents the trachea, and generation 15 is the start of the alveolar region); and

[0035] **Fig 7**: **Fig. 7** shows Lagrangian simulation results displaying the various airway surface liquid (ASL) concentrations for various tracheal velocity and daily mucus production rates.

[0036] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of illustrative embodiments with reference to the accompanying drawings, which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE ILLUSTRATIVE EMBODIMENTS

[0037] In order to provide a clear and consistent understanding of the terms used in the present specification, a number of definitions are provided below.

[0038] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one", but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one". Similarly, the word "another" may mean at least a second or more.

[0039] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "include" and "includes") or "containing" (and any form of containing, such as "contain" and "contains"), are inclusive or open-ended and do not exclude additional,

unrecited elements or method steps.

[0040] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0041] The term "about" is used to indicate that a value includes an inherent variation of error for the device or the method being employed to determine the value.

[0042] The term "antineoplastic agent", as used herein, is understood as being an agent that prevents the development, growth or proliferation of malignant cells.

[0043] The term "cancer", as used herein, is understood as referring to the uncontrolled growth of abnormal cells.

[0044] The term "subject" or "individual", as used herein, is understood as referring to a human or animal in need of medical treatment.

[0045] The term "respiratory tract", as used herein, is understood as referring to the regions including the oral and nasal-pharyngeal tracheo-bronchial, and pulmonary regions.

[0046] The term "pulmonary region", as used herein, is understood as referring to the region including the upper and lower bronchi, bronchioles, terminal bronchioles, respiratory bronchioles and alveoli.

[0047] The term "marker molecule", as used herein, is understood as referring to a molecule used in the diagnosis of a condition of the respiratory tract and/or lungs.

[0048] As used herein, it is to be understood that when a "range" or a "group of substances" is mentioned, or when a particular characteristic, whether physical or chemical such as temperature, concentration, time and the like is mentioned, the present invention relates to, and explicitly incorporates herein each and every specific member and combination of sub-ranges or sub-groups therein whatsoever. Thus, any specified range or group is to be understood as a shorthand way of referring to each and every member of a range or group individually as well as each and every possible sub-ranges or sub-groups encompassed therein; and similarly with respect to any sub-ranges or sub-groups therein. Thus, for example, with respect to reaction time, a time of 1 minute or more is to be understood as specifically incorporating herein each and every individual time, as well as sub-ranges above 1 minute, such as for example 3 to 15 minutes, 1 minute to 20 hours, 1 to 3 hours, 16 hours, 3 hours to 20 hours etc. The same applies to other parameters such as for example concentrations, temperatures, etc.

[0049] The present invention broadly relates to a dry powder drug delivery vehicle suitable for inhalation aerosol therapy. More specifically, the present invention relates to inhalable aerosol dry powders comprising drug loaded nanoparticles. In an embodiment, the present invention relates to inhalable aerosol dry powders comprising lipoplex doxorubicin nanoparticles. In a further embodiment, the present invention relates to inhalable aerosol dry powders comprising doxorubicin-loaded cyanoacrylate nanoparticles. The inhalable aerosol powders further comprise a carrier and may be conveniently manufactured using a spray-freeze drying process. The spray-freeze drying process offers the advantage of producing highly porous aerosol particles (friable powders suitable for inhalation) having large physical diameters, but having small aerodynamic diameters. In a particular embodiment, the inhalable aerosol dry powders comprise lactose as a carrier. The inhalable aerosol dry powders of the present invention have characteristic aerodynamic properties. In a particular embodiment of the present invention, the mass median aerodynamic diameter (MMAD) of the doxorubicin-loaded DMPG-based powders was determined to be about 1.74 ± 0.2 μm with a geometric standard deviation (GSD) of about 3.5 ± 0.1, while the mass median aerodynamic diameter (MMAD) of the doxorubicin-loaded cyanoacrylate-based powder was determined to be about 3.4 ± 0.2 μm with a geometric standard deviation (GSD) of about 3.1 ± 0.1.

[0050] Because of ionic interactions between the cationic doxorubicin molecules and the anionic nature of DMPG, doxorubicin and DMPG tend to form a doxorubicin-DMPG lipoplex. In contrast to the cyanoacrylate nanoparticulate powders of the present invention, the lipoplex comprising powders of the present invention display high levels of electrostatic interactions making handling and deagglomeration difficult.

[0051] *In vitro* cytotoxicity studies using an XTT assay system were performed on the H460, A549, DU145 and SK-NSH cancer cell lines with plain doxorubicin as well as with the powder formulations. The LC_{50} for the doxorubicin-DMPG powders remains essentially identical to plain doxorubicin with respect to cell lines H460 and A549, respectively. The LC_{50} for the doxorubicin loaded cyanoacrylate nanoparticle powder formulations followed the same cytotoxic trend as plain doxorubicin in cell lines H460 and SK-NSH but decreased about a 3-fold in cell lines A549 and DU145.

[0052] An aerosol deposition simulation using a 20 mg powder dose of a doxorubicin-loaded cyanoacrylate nanoparticle powder formulation allowed for a prediction of the regional deposition and airway surface liquid concentration (ASL) of doxorubicin from the powder dose. From a 20 mg powder dose, the peak concentration predicted by the simulation occurs in generation 1 and is 1.85 μg/ml, while the low of 0.1 μg/ml is predicted to occur in generation 14. In order to improve patient compliance with respect to inhalation aerosol therapy, it is important that as much of the therapeutic agent is delivered as quickly as possible (*i.e.* requiring the least amount of repetitive inhalations). In an embodiment, the present invention relates to cyanoacrylate-based nanoparticles comprising a therapeutically effective amount of the therapeutic agent to be administered and which essentially do not suffer from drug leakage upon minor dilution (*i.e.* drug dissociation from the nanoparticles; Table 2). In the case of a doxorubicin solution (1mg/mL), from about 70% to about 95% of the doxorubicin can be entrapped within polybutylcyanoacrylate-based nanoparticles.

[0053] The present invention is illustrated in further detail by the following non-limiting examples.

[0054] Materials and Methods

[0055] Doxorubicin hydrochloride (DXR) was purchased from Sigma (St. Louis, MO, USA); Doxorubicin hydrochloride (DXR) injection USP 2mg/mL was obtained from Novopharm (Toronto, ON, Canada); 1,2-Dimyristoyl-sn-glycero-phosphoglycerol sodium salt (DMPG) was obtained from Genzyme Pharmaceutics (Cambridge, MA, U.S.A); *n*-Butyl cyanoacrylate was a gift of Henkel Loctite, (Dublin, Ireland); Alternatively, *n*-Butyl cyanoacrylate monomer may be prepared by the Knoevenagal condensation reaction between *n*-butyl cyanoacetate and formaldehyde followed by purification by distillation; Dextran 70 was obtained from ScienceLab.com, Inc. (Kingwood, TX 77339 USA); Lactose monohydrate was obtained from Pharmatose 325M DMV International (The Netherlands).

[0056] Human non-small cell lung cancer (NSCLC) H460 and A549 cell lines , prostate cancer DU145 cell line and neuroblastoma SK-NSH and SK-NMC cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cytotoxicity was analyzed using a cell proliferating XTT kit (Roche Molecular Biochemical, Laval, Quebec, Canada).

EXAMPLE 1
Lipid Nanoparticle Powders

[0057] Three formulations were created using the materials and quantities shown in Table 1. For powder formulation L1, DMPG and doxorubicin hydrochloride were added to a lactose solution in a 15 ml test tube. The suspension was vortexed on a wrist-activated shaker for 2 x 1 minute, followed by a one hour equilibration period at 23 °C in darkness. The spray-freeze drying procedure was subsequently applied to the suspension.

[0058] Powder formulation L2 was produced by adding DMPG to the lactose solution and sonicating the mixture for 30 minutes in a bath. The doxorubicin solution was then added to the mixture and sonication continued for an additional one hour. The resulting suspension was filtered through a 0.45 μm filter and was left to equilibrate for one hour at 23 °C. The suspension was then submitted to spray freeze-drying (SFD).

[0059] Powder formulation L3 was produced similarly to L2, except that prior to the addition of the doxorubicin solution to the DMPG/lactose suspension, doxorubicin hydrochloride was dissolved in a saline solution and the pH adjusted to 3 using HCl.

TABLE 1

Contents of the Lipoplex-based powder formulations.

Powder	DXR (mg)	DMPG (mg)	Lactose (12% aq.) (ml)
L1	10	70	12.5
L2	7*	42	10.5
L3	10	60	10.5**

*Doxorubicin hydrochloride was used: 3.5 ml of a solution containing 2 mg/ml DXR and 9 mg/ml NaCl.
**An additional 5 ml of 0.9 % NaCl adjusted with HCl to pH = 3 was utilized.

EXAMPLE 2

Butyl Cyanoacrylate Nanoparticle Powders

[0060] The polymeric butyl cyanoacrylate nanoparticles utilized in this study were created utilizing previously published procedures [DeVerdiere, A.C., Dubernet, C., Nemati, F., Soma, E., Appel, M., Bernard, S., Puisieux, F., Couvreur, P. 1997; *Reversion of multidrug resistance with polyalkylcyanoacrylate nanoparticles: towards a mechanism of action.* British J. of Cancer. 76: 198-205]. Butyl cyanoacrylate (BCA) monomer (234 µl) was added drop-wise to a continuously stirred (800 rpm) solution comprising citric acid (14.4 ml of a 0.5% solution), dextran 70 (234 mg), and doxorubicin (9 ml; 2 mg/ml). The suspension was agitated for an additional four hours at 23 °C in darkness. The suspension was subsequently centrifuged at 14 000 rpm for one hour at 4 °C (Allegra 21R, Beckman Coulter, CA, USA). The so-obtained pellets were redispersed in a 12% lactose solution comprising 0.5% dextran 70 to finally provide a doxorubicin-nanoparticle suspension (11 ml) which was subsequently submitted to spray freeze-drying (SFD). The spray-freeze drying procedure provides for very friable powders, appropriate for administration by inhalation (*i.e.* respiration).

[0061] Two alternate cyanoacrylate nanoparticle formulations were also produced for comparative purposes. A powder comprising unloaded cyanoacrylate nanoparticles was created following the procedure described hereinabove with the exception of replacing the doxorubicin (9 ml; 2 mg/ml) solution with a HCl solution (9 ml; 0.001N).

[0062] A powder comprising unloaded cyanoacrylate nanoparticles and further comprising a dye in the carrier phase was produced. The unloaded polymeric nanoparticles were added to an 11 ml solution comprising 12% lactose, 0.5% dextran 70 and 0.3 % methylene blue. The resulting suspension was subsequently submitted to spray freeze-drying (SFD).

EXAMPLE 3
Spray Freeze-Drying Procedure

[0063] Spray freeze-drying was used to manufacture powders from the corresponding suspensions. A two-fluid nozzle (Spraying Systems Co., Wheaton, IL, USA) utilizing gaseous nitrogen at a flow rate of 0.6 scfm (standard cubic feet per minute) was employed to atomize the suspensions, which were supplied at a flow rate of 37 ml/min using a peristaltic pump (CTP-A, Chem-Tech, Punta Gorda, FL, USA). The nozzle was placed about 15 cm above a flask (600 ml) comprising from about 300 to about 400 ml of liquid nitrogen. Following spraying, the flask contents were transferred into a Pyrex vacuum beaker, and the liquid nitrogen was allowed to evaporate. The vacuum container was attached to a freeze dry system (Freezone 4.5, Labconco Corp., Kansas City, MO, USA) operating at 0.004 mbarr with the collector at -52 °C. The powder in the flask was held at subzero temperature for an initial 7 hours, followed by a period of 41 hours at 23 °C. After 48 hours, the powder was collected and stored in a sealed vial at 4 °C.

EXAMPLE 4
Aerodynamic Characterization of Powders

[0064] Size distribution measurements of powder particles were accomplished using an Anderson Cascade Impactor (Graseby Anderson, Smyma, GA, USA) functioning at an air flow rate of 60 liters per minute with plate cut-off

diameters adjusted as previously described [Nichols, S. C., Brown, D. R., Smurthwaite, M. 1998; *New concept for the variable flow rate Anderson impactor and calibration data*. Journal of Aerosol Medicine **11** Supplement 1, S133–S138]. Deagglomeration of the powder was achieved using a proprietary inhaler utilizing cyclonic action as well as mechanical impaction as dispersion mechanisms [US Patent Application no. 20040107963]. The impactor plates were sprayed with 316 Silicone Release Spray (Dow Corning, Midland, MI, USA) prior to use. Subsequent to powder loading, each plate was washed with methanol and the extracts were assayed for doxorubicin using an ultraviolet spectrophotometer (UV absorbance at λ=479 nm, Diode Array Spectrophotometer, model 8452A, Hewlett Packard, Tulsa, OK, USA). The methanol extracts comprising the powder containing doxorubicin-loaded cyanoacrylate nanoparticles were filtered prior to ultraviolet measurements in order to remove any dextran precipitate.

[0065] Respirable or fine-particle fraction measurements were performed on the powder containing doxorubicin-loaded cyanoacrylate nanoparticles. A pulmonary waveform generator (MH Custom Design & MFG., Salt Lake City, UT, USA) was utilized to drive inhalational flow through a Respirguard bacterial/viral filter (#303, Totowa, NJ, USA) that was serially connected to an idealized throat [Stapleton, K. W., Guentsch, E., Hoskinson, M. K. & Finlay, W. H. 2000; *On the suitability of k-epsilon turbulence modeling for aerosol deposition in the mouth and throat: a comparison with experiment*, J. Aerosol Sci. 31:739-749. DeHaan, W. H. & Finlay, W. H. 2001; *In vitro monodisperse aerosol deposition in a mouth and throat with six different inhalation devices*, J. Aerosol Med. 14:361-367], and the above-mentioned inhaler. The inhalation time was 4 seconds, and the waveform supplied was a ramped 60 l/min square wave. The throat was coated with silicone release spray prior to use. Following powder inhalation, the filter was washed with methanol and the extracts assayed for doxorubicin. The percentage of the total dose contained in the filter is considered to be the respirable fraction.

EXAMPLE 5
Activity Testing

[0066] A colorimetric cell proliferating XTT assay system was utilized to determine the cytotoxicity of the various doxorubicin configurations on the H460,

A549, DU145, SK-NSH, and SK-NMC cancer cell lines. Cells were grown in a humidified 5% carbon dioxide atmosphere at 37°C using a 96-well microplate, with each well comprising about 5000 cells immersed in 100 µl of 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were allowed to adhere for 14 hours. The media in each well was subsequently replaced with a mixture of 2% fetal bovine serum comprising serial dilutions of the tested doxorubicin configurations. Following a 48 hour incubation period, 50 µl of the XTT labeling mixture was added to each well. The microplate was incubated for a further 4 hours. A Benchmark microplate reader (BioRad Laboratory, Mississauga, Ontario, Canada) operating with a 492 nm optical filter and a 650 nm reference wavelength was utilized to measure the spectrophotometrical absorbance of each well. The fraction of viable cells was calculated (unity subtract the optical density fraction of treated cells to untreated cells). Each arrangement had a minimum of two measurements.

EXAMPLE 6

Deposition Simulation

[0067] A Lagrangian deposition simulation developed by Finlay & Stapleton (Finlay, W. H. & Stapleton, K. W.; "*The effect on regional lung deposition of coupled heat and mass transfer between hygroscopic droplets and their surrounding phase*", J. Aerosol Science 26:655-670, 1995) was used in conjunction with an airway surface liquid (ASL) model [Lange, C. F., Hancock, R. E. W., Samuel, J., and Finlay, W. H. 2001; *In vitro aerosol delivery and regional airway surface liquid concentration of a liposomal cationic peptide*, J. Pharm. Sci. 90:1647-1657] to predict the airway surface liquid concentration of doxorubicin from a 20 mg dosage of powder containing the doxorubicin-loaded cyanoacrylate nanoparticles. The deposition simulation utilized the mass median aerodynamic diameter and geometric standard deviation as measured using the cascade impactor, as well as mucous production rates and tracheal mucous velocities of 5 ml/day & 40 ml/day and 5 mm/min & 15 mm/min, respectively. A low tracheal velocity coupled with a high mucous production rate produces the minimum doxorubicin concentration prediction, while the converse arrangement produces the maximum doxorubicin concentration prediction.

EXAMPLE 7

Doxorubicin-DMPG Powder

[0068] Upon spray freeze-drying the formulations L1, L2, and L3 as described in Table 1, about 130 mg of spray freeze-dried powder of each was obtained. Upon resuspension in isotonic saline, the nanoparticle mean diameter, by volume, was measured to be 294 ± 122 nm. A powder containing DMPG, lactose and NaCl (in the same ratio as powder L2) was reconstituted in isotonic saline. The mean particle diameter was measured to be 141 nm. Nanoparticle size measurements on the doxorubicin-DMPG suspensions were typically bimodal, showing a first peak near 400 nm and a second peak > 1μm. It is assumed that the larger peak corresponds to a measurement of agglomerated DMPG-doxorubicin particles.

[0069] The doxorubicin-DMPG suspensions show a shift towards larger nanoparticle sizes when compared to plain DMPG in ionic media, which would be indicative of an interaction between doxorubicin and DMPG. The structures resulting from such an interaction may either be liposomes, micelles, bicelles, or any other doxorubicin-DMPG adduct. The formation of a doxorubicin-DMPG lipoplex is likely to occur due to the ionic interaction between the anionic nature of DMPG and the cationic doxorubicin molecules. In addition, both molecules are lipophilic.

[0070] DMPG is known to form bicelles in solution with a specific threshold ionic strength [Meyer, H.M., Richter, W., Rettig, W., Stumpf, M. 2001; *Bilayer fragments and bilayered micelles (bicelles) of 1,2-dimyristoylphosphatidylglycerol (DMPG) are induced by storage in distilled water at 4°*. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 183-185: 495 – 504]. Suspensions L2 and L3, prior to spray freeze-drying, displayed pellet formation upon centrifugation, which further indicates a doxorubicin-DMPG interaction. Both the L2 and L3 suspensions comprise 0.9 % sodium chloride. The L1 suspension, however, did not comprise any NaCl and showed no pellet formation upon centrifugation prior to spray freeze-drying. Evidence suggests that an ionic solution may be required for the formation of a doxorubicin-DMPG lipoplex. It is possible and indeed likely that the ionic strength of bodily fluids, such as the airway surface fluid, may be strong enough to facilitate doxorubicin-DMPG lipoplex

formation.

[0071] All the DMPG-based powders of the present invention were found to be adhesive. The handling of the powders proved to be difficult and the effectiveness of the inhaler may therefore be adversely affected. Utilizing additional lactose in the formulations may decrease the auto-adhesive nature of the powder, but a resulting decrease in doxorubicin concentration may also occur.

EXAMPLE 8
Doxorubicin-Cyanoacrylate Powder

[0072] Spray freeze-drying the previously prepared doxorubicin-nanoparticle suspensions (11 ml) produced about 150 mg of spray freeze-dried powder per suspension. Figure 1 compares the nanoparticle size distributions of doxorubicin loaded cyanoacrylate powder to that of unloaded cyanoacrylate powder. The nanoparticle diameter comprising the median volume was 162.4 ± 9.9 nm (mean ± s.d., n=3) for the unloaded nanoparticles, and 391.8 ± 13.7 nm for the doxorubicin-loaded nanoparticles, which differ significantly from each other ($P<0.01$, Student t-test). The differences in the size distribution can be attributed to the incorporation of doxorubicin into the cyanoacrylate matrix. Furthermore, the particle distribution for the doxorubicin-loaded cyanoacrylate nanoparticles is slightly bimodal. In an embodiment of the present invention, about 850 μg of doxorubicin were loaded into nanoparticles per milliliter of colloidal suspension, which correlates to about an 85% loading efficiency. This loading efficiency was achieved by mixing doxorubicin hydrochloride (1 mg) with lactose (120 mg), dextran 70 (10 mg) and about 10 mg of polymer, all in water. Following the removal of the water by spray freeze-drying, a dry powder was obtained (140 mg) comprising 850 μg of doxorubicin loaded in nanoparticles (*i.e.* roughly 1 mg of doxorubicin loaded into nanoparticles per 140 mg of dry powder).

[0073] Liposomally encapsulated therapeutic agents often display a decrease in encapsulation efficiency when the suspension is diluted. Liposome formation is typically dependent on the ionic strength of the solution. Therefore, a decrease in ionic strength often results in a decrease in encapsulation efficiency. This is an undesirable property when the desired therapeutic effect depends on the

drug configuration. For this reason, the similar potential for doxorubicin to disassociate from the cyanoacrylate nanoparticles upon dilution was tested. A suspension containing doxorubicin-loaded nanoparticles, prior to spray freeze-drying, was diluted with isotonic saline and centrifuged to determine the influence of dilution on doxorubicin leakage. The results are presented below in Table 2. A 100-fold dilution resulted in a 33% loss of doxorubicin from the polymeric nanoparticles. Doxorubicin-loaded cyanoacrylate nanoparticle disassociation is therefore a weak function of nanoparticle concentration.

TABLE 2

The effect of dilution on doxorubicin encapsulation in butyl cyanoacrylate nanoparticles.

Original Encapsulation (%)	Dilution	Encapsulation upon Dilution (%)
90.4	5 x	83.1
75.6	5 x	85.9
75.2	10 x	64.2
75.6	20 x	77.5
75.2	50 x	50.2
75.2	100 x	50.2

[0074] A number of problems were encountered when attempting to increase the doxorubicin loading in the cyanoacrylate powder. Primarily, it was difficult to achieve high concentrations of doxorubicin in the nanoparticles without also concomitantly increasing the size of the nanoparticles. This problem is amplified when formulating powders also comprising a carrier (*i.e.* lactose) since the amount of doxorubicin per mg of powder decreases (*i.e.* lowered concentration). In order to address this issue, a lower quantity of carrier (*i.e.* lactose) could be utilized in the formulation. However, this results in a powder with increased electrostatic interaction, which in turn results in increased difficulty of handling and decreased deagglomeration with the anticipated associated negative effects on aerosol properties.

EXAMPLE 9

Aerodynamic Characterization

[0075] The mass median aerodynamic diameters (MMAD) and geometric standard deviation (GSD) of the doxorubicin-DMPG powders are displayed below in Table 3.

TABLE 3

Aerodynamic properties of doxorubicin-DMPG powders

Sample (mg)	Feeding Time (s)	FPF (%)	MMAD (μm)	GSD
11.2	30	77.9	1.86	3.4
12.0	20	65.3	1.61	3.7
12.0	30	62.4	1.64	3.5
11.8	30	70.2	1.48	3.5
13.5	20	59.2	2.11	3.4

[0076] Due to the electrostatic nature of these powders, the loading rate of the inhaler was limited to 0.4 – 0.6 mg/sec over a period of 20 – 30 seconds. The average MMAD for the doxorubicin-DMPG powders was 1.74 μm ± 0.2 μm (mean ± s.d.) while the geometric standard deviation was 3.5 ± 0.1. The powder dispersion capabilities of the inhaler are dependent on the powder loading rate as well as the electrostatic properties of the powder. In the present case, the MMAD is deceptively low, due to the impractically long powder dispersion time. The powder is also quite polydisperse, likely due to the agglomeration of particles.

[0077] The doxorubicin-loaded cyanoacrylate nanoparticle containing powder had a significantly higher mass median aerodynamic diameter (MMAD) compared to the doxorubicin-DMPG nanoparticle containing powder, while having a similar geometric standard deviation (GSD) (3.5 ± 0.1). The cascade impaction results are displayed below in Table 4.

TABLE 4

Aerodynamic properties of the doxorubicin-loaded cyanoacrylate powders.

Sample (mg)	Feeding Time (s)*	$FPF_{<5.6\mu m}$ (%)	MMAD (μm)	GSD
20.2	8	47.3	3.66	3.0
18.8	8	40.4	3.64	3.1
18.9	8	41.0	3.24	3.0
18.9	8	38.9	3.27	3.3
20.0	8	40.6	3.50	3.1
18.0	8	39.3	3.14	3.3

**The samples were divided approximately in half and each portion was fed over a 4 second period.*

[0078] The fine particle fraction (FPF), defined as the mass fraction of aerosol with a MMAD < 5.6 μm), was measured to be 41.3% ± 3.0% (n=5). The higher MMAD was obtained using a more realistic dispersion time: powder doses (about 20 mg) were loaded by splitting the sample in half, then loading each 10 mg sample over a 4 second period. This method corresponds to a powder loading rate of 2.5 mg/sec.

[0079] An accurate *in vitro* prediction of the amount of therapeutic dose reaching the lungs can be obtained by using the idealized throat (the Alberta geometry) serially connected to the breathing machine. The dosage loading time was set at 4 seconds, consistent with the cascade impaction measurements. The fraction of doxorubicin-loaded cyanoacrylate powder loaded into the inhaler that reached the filter (the "lungs") downstream of the mouth-throat was measured to be 39.2% ± 3.6% (n=4).

[0080] To determine if the doxorubicin-loaded cyanoacrylate nanoparticles were uniformly distributed among the aerosol particles, the aerosol size distributions of the methylene blue dyed powder and the doxorubicin-loaded nanoparticle powder were compared. The suspension containing the methylene

blue, prior to spray freeze-drying, was centrifuged. The supernatant contained 98% of the dye, which indicates that virtually all of the dye adsorbs to the lactose. The observed MMAD and geometric standard deviation of the nanoparticle containing powder loaded with methylene blue were essentially identical to those observed for the doxorubicin-loaded cyanoacrylate nanoparticle containing powder, which is indicative of the nanoparticles being uniformly distributed throughout the aerosol particles.

EXAMPLE 10
Cytotoxicity Study

[0081] *In vitro* cytotoxicity studies were performed on plain doxorubicin as well as on the powder formulations. The LC_{50} for doxorubicin-DMPG remains essentially identical to plain doxorubicin with respect to cell lines H460 and A549, respectively. The DMPG formulation would be a noteworthy improvement over plain doxorubicin, if the toxicity on healthy cells was significantly reduced. However, the possible reduction of the toxicity of doxorubicin on healthy cells, due to a lipoplex formation with DMPG, was not investigated herein.

[0082] The results of the cytotoxicity study of plain doxorubicin and doxorubicin-loaded cyanoacrylate nanoparticle containing powders on cell lines H460, A549, DU145, and SK-NSH are illustrated in Figures 2-5. In the Figures, NPD-13 and NPD-14 indicate separate batches of doxorubicin-loaded cyanoacrylate nanoparticle containing powders differing in their specific drug concentration (the NPD-14 batch comprising 20% more doxorubicin). Nevertheless, normalized values are used for comparisons. The polymeric nanoparticle doxorubicin formulation followed the same cytotoxic trend as plain doxorubicin in cell lines H460 and SK-NSH. However, in the A549 cell line, the LC_{50} decreased from about 1.2 μg/ml to about 0.32 μg/ml in going from plain doxorubicin to the polymeric nanoparticle doxorubicin formulation. A similar observation could be made in the DU145 cell line in which the LC_{50} decreased from about 0.8 μg/ml to about 0.32 μg/ml in going from plain doxorubicin to the polymeric nanoparticle doxorubicin formulation. A significant advantage of placing doxorubicin in a polymeric matrix is that doxorubicin resistance in the A549 and DU145 cell lines is partly reversed. This is suggestive of a possible mechanism involving nanoparticle/cell interaction(s), which could potentially help

overcome multi-drug resistance expressed by the A549 cell line [Liu, L. Z., Qian G. S., Zhou X., D. 2003; *Expression of a new lung cancer drug resistance-related gene in lung cancer tissues and lung cancer cell strains.* Chinese Journal of Cancer. 22(2): 171-174. Trussardi A., Poitevin G., Gorisse M., Faroux M., Bobichon H., Delvincourt C., Jardillier J. 1998; *Sequential overexpression of LRP and MRP but not P-gp 170 in VP16-selected A549 adenocarcinoma cells.* Int. J. Oncol. 13(3):543-8]. A logical follow-up study may include the application of specific multi-drug resistance protein inhibitors so as to examine the effect in combination with doxorubicin-loaded nanoparticles.

[0083] Current chemotherapy utilizing doxorubicin dictates an intravenous delivery of about 60 - 75 mg/m^2. Since the average human has about 1.8 m^2 of body surface area, such a delivery provides for an intravenous doxorubicin dosage of about 130 mg. If delivery is directly to the lungs, then the dosage can be reduced by a factor of 24, [Sharma, S., White, D., Imondi, A.R., Placke, M.E., Vail, D.M., Kris, M.G. 2001; *Development of inhalational agents for oncologic use.* J Clinical Oncology. 19: 1839 – 1847] which would decrease the doxorubicin dosage to about 5.4 mg. Considering the approximately 3-fold decrease in the LC_{50} concentration in going from plain doxorubicin to the polymeric nanoparticle doxorubicin formulation, as observed in the A549 cell line, a patient would only require an inhalation dosage comprising 1.8 mg of doxorubicin. With the present formulation, this would correspond to a total powder dosage of 240 mg. Moreover, systemic side-effects will likely be significantly reduced due to the 96-fold decrease in doxorubicin dosage (1.8 mg vs. 130 mg) associated with the lung delivery of the polymeric nanoparticle formulations of the present invention.

EXAMPLE 11

Lagrangian Deposition Simulation

[0084] An aerosol deposition simulation was performed to provide a prediction of the regional deposition of the nanoparticle comprising powder particles based on the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) of the powder particles as measured by the cascade impactor. A 20 mg powder dose comprising 7.5 μg of doxorubicin per mg of powder (similar to that delivered by the inhaler testing shown in Table 4), produced the

regional quantities shown in Figure 6. The maximum and minimum airway surface liquid (ASL) concentrations for the tracheobronchial region can be predicted as shown in Figure 7. It is advantageous to know the airway surface liquid concentration since lung cancers originate or metastasize to the epithelium and since the nanoparticle containing powder dissolves in the airway surface liquid prior to the nanoparticles diffusing through the airway surface liquid into the tumor/ epithelium. For a 20 mg powder dose, the peak concentration predicted by the simulation occurs in generation 1 and is 1.85 μg/ml, while the low of 0.1 μg/ml is predicted to occur in generation 14 (Figure 7). Generation, as used in the figures, refers to lung generation (*i.e.* how many branches of the lung were reached). Thus the trachea represents generation 0, the main bronchi generation 1, the lobar bronchi generation 2, etc. The deeper the region of the lung, the higher the generation number. Intravenous doxorubicin treatment produces a peak plasma concentration of 2.9 μg/ml [Muller, I., Jenner, A., Bruchelt, G., Niethammer, D., Halliwell, B. 1997; *Effect of concentration on the cytotoxic mechanism of doxorubicin – apoptosis and oxidative DNA damage*. Biochemical and Biophysical Research Communications. 230: 254-257] from a total doxorubicin dosage ranging from about 108 mg to 135 mg. Multiple inhalational dosages of the nanoparticle containing powder formulations of the present invention may thus produce pharmacologically relevant concentrations of doxorubicin in the tracheobronchial region. Given the aerodynamic properties of the nanoparticle containing powder formulations of the present invention, the number density of particles in the air stream, and the inhalational flow rate, the simulation predicts essentially instantaneous maximum airway surface liquid concentrations (Figure 7). The doxorubicin airway surface liquid concentration will decrease as the species convect and diffuse through the epithelium. Consequently, the initial doxorubicin concentration is a first-order estimate of the doxorubicin concentration at a tumor site in or on the epithelium. The simulation suggests that one of the benefits of localized drug delivery may be efficacious drug concentrations at tumor sites arising from lower total dosages.

[0085] Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified without departing from the spirit and nature of the subject invention as defined in the appended claims.

What is claimed is:

1. A pharmaceutical formulation comprising spray-freeze dried powder, said powder comprising nanoparticles loaded with at least one active principle, said powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region, said formulation being administered by aerosol inhalation to the lung.

2. The pharmaceutical formulation of claim 1, wherein said maximum airway surface liquid concentrations of the active principle are provided at least prior to deposition and dissolution of the powder in generation 2 of the lung.

3. The pharmaceutical formulation of claim 2, wherein said maximum airway surface liquid concentrations of the active principle are provided at least following deposition and dissolution of the powder at about generation 1 of the lung.

4. The pharmaceutical formulation of claim 2, wherein said generation 2 is representative of the lobar bronchi of the lung.

5. The pharmaceutical formulation of claim 3, wherein said generation 1 is representative of the main bronchi of the lung.

6. The pharmaceutical formulation of claim 1, wherein said active principle is selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers, imaging enhancers and combinations thereof.

7. The pharmaceutical formulation of claim 6, wherein the drug is an antineoplastic agent selected from the class of anthracyclines.

8. The pharmaceutical formulation of claim 7, wherein the anthracyclines comprise doxorubicin, epirubicin, daunorubicin, idarubicin and corresponding salts.

9. The pharmaceutical formulation of claim 8, wherein the anthracycline is doxorubicin or doxorubicin hydrochloride.

10. The pharmaceutical formulation of claim 6, further comprising a carrier selected from the group consisting of lactose, dextran, mannitol, trehalose, glucose, fructose and saccharose.

11. The pharmaceutical formulation of claim 10, wherein the carrier is lactose.

12. The pharmaceutical formulation of claim 1, wherein the nanoparticles are generated using a cyanoacrylate monomer capable of forming a polymeric matrix.

13. The pharmaceutical formulation of claim 12, wherein the cyanoacrylate monomer is selected from the group consisting of butyl cyanoacrylate, isobutyl cyanoacrylate and octyl cyanoacrylate.

14. The pharmaceutical formulation of claim 10, wherein the powder is characterized by a mass median aerodynamic diameter (MMAD) ranging from about 0.5 to about 10.0 μm.

15. The pharmaceutical formulation of claim 14, wherein the powder is further characterized by a geometric standard deviation (GSD) ranging from about 1.0 to about 4.0.

16. The pharmaceutical formulation of claim 8, wherein the anthracycline loaded nanoparticles have a size ranging from about 1.0 nm to about 1000 nm.

17. A drug delivery vehicle for inhalation aerosol therapy comprising spray-freeze dried powder, said powder comprising nanoparticles loaded with at least one active principle, said powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region.

18. The drug delivery vehicle of claim 17, wherein said maximum airway surface liquid concentrations of the active principle are provided at least prior

to deposition and dissolution of the powder in generation 2 of the lung.

19. The drug delivery vehicle of claim 18, wherein said maximum airway surface liquid concentrations of the active principle are provided at least following deposition and dissolution of the powder at about generation 1 of the lung.

20. The drug delivery vehicle of claim 17, wherein said generation 2 is representative of the lobar bronchi of the lung.

21. The drug delivery vehicle of claim 19, wherein said generation 1 is representative of the main bronchi of the lung.

22. The drug delivery vehicle of claim 17, wherein said active principle is selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers, imaging enhancers and combinations thereof.

23. The drug delivery vehicle of claim 22, wherein the drug is an antineoplastic agent selected from the class of anthracyclines.

24. The drug delivery vehicle of claim 23, wherein the anthracyclines comprise doxorubicin, epirubicin, daunorubicin, idarubicin and corresponding salts.

25. The drug delivery vehicle of claim 24, wherein the anthracycline is doxorubicin or doxorubicin hydrochloride.

26. The drug delivery vehicle of claim 22, further comprising a carrier selected from the group consisting of lactose, dextran, mannitol, trehalose, glucose, fructose and saccharose.

27. The drug delivery vehicle of claim 26, wherein the carrier is lactose.

28. The drug delivery vehicle of claim 17, wherein the nanoparticles are generated using a cyanoacrylate monomer capable of forming a polymeric matrix.

29. The drug delivery vehicle of claim 28, wherein the cyanoacrylate monomer is selected from the group consisting of butyl cyanoacrylate, isobutyl

cyanoacrylate and octyl cyanoacrylate.

30. The drug delivery vehicle of claim 26, wherein the powder is characterized by a mass median aerodynamic diameter (MMAD) ranging from about 0.5 to about 10.0 μm.

31. The pharmaceutical formulation of claim 30, wherein the powder is further characterized by a geometric standard deviation (GSD) ranging from about 1.0 to about 4.0.

32. The pharmaceutical formulation of claim 24, wherein the anthracycline loaded nanoparticles have a size ranging from about 1.0 nm to about 1000 nm.

33. A method of formulating a powder containing nanoparticles for inhalation aerosol delivery to the lung, the method comprising the steps of:

mixing a cyanoacrylate monomer with a liquid carrier and at least one active principle to create a suspension comprising nanoparticles loaded with said at least one active principle; and

submitting the suspension to spray freeze-drying producing carrier particles suitable for aerosol delivery to the lung.

34. The method of claim 33, wherein the carrier particles provide for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the carrier particles in the airway surface liquid following deposition of the carrier particles throughout the tracheobronchial region, said carrier particles being administered by aerosol inhalation to the lung.

35. The method of claim 34, wherein said maximum airway surface liquid concentrations of the active principle are provided at least prior to deposition and dissolution of the carrier particles in generation 2 of the lung.

36. The method of claim 35, wherein said maximum airway surface liquid concentrations of the active principle are provided at least following deposition and dissolution of the carrier particles at about generation 1 of the lung.

37. The method of claim 35, wherein said generation 2 is representative of the lobar bronchi of the lung.

38. The method of claim 36, wherein said generation 1 is representative of the main bronchi of the lung.

39. The method of claim 33, wherein said active principle is selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers, imaging enhancers and combinations thereof.

40. The method of claim 39, wherein the drug is an antineoplastic agent selected from the class of anthracyclines.

41. The method of claim 40, wherein the anthracyclines comprise doxorubicin, epirubicin, daunorubicin, idarubicin and corresponding salts.

42. The method of claim 41, wherein the anthracycline is doxorubicin or doxorubicin hydrochloride.

43. The method of claim 39, wherein the carrier is selected from the group consisting of lactose, dextran, mannitol, trehalose, glucose, fructose and saccharose.

44. The method of claim 43, wherein the carrier is lactose.

45. The method of claim 33, wherein the cyanoacrylate monomer is selected from the group consisting of butyl cyanoacrylate, isobutyl cyanoacrylate and octyl cyanoacrylate.

46. A method of treating lung cancer in a patient comprising the step of administering to said patient a formulation comprising spray-freeze dried powder, said powder comprising nanoparticles loaded with at least one active principle, said powder providing for rapid maximum airway surface liquid concentrations of the active principle upon dissolution of the powder in the airway surface liquid following deposition of the powder throughout the tracheobronchial region, said formulation being administered by aerosol inhalation to the lung.

47. The method of claim 46, wherein said maximum airway surface liquid concentrations of the active principle are provided at least prior to deposition and dissolution of the powder in generation 2 of the lung.

48. The method of claim 47, wherein said maximum airway surface liquid concentrations of the active principle are provided at least following deposition and dissolution of the powder at about generation 1 of the lung.

49. The method of claim 47, wherein said generation 2 is representative of the lobar bronchi of the lung.

50. The method of claim 48, wherein said generation 1 is representative of the main bronchi of the lung.

51. The method of claim 46, wherein said active principle is selected from the group consisting of drugs, vaccines, virus vectors, marker molecules, tracers, imaging enhancers and combinations thereof.

52. The method of claim 51, wherein the drug is an antineoplastic agent selected from the class of anthracyclines.

53. The method of claim 52, wherein the anthracyclines comprise doxorubicin, epirubicin, daunorubicin, idarubicin and corresponding salts.

54. The method of claim 53, wherein the anthracycline is doxorubicin or doxorubicin hydrochloride.

55. The method of claim 51, further comprising a carrier selected from the group consisting of lactose, dextran, mannitol, trehalose, glucose, fructose and saccharose.

56. The method of claim 55, wherein the carrier is lactose.

57. The method of claim 46, wherein the nanoparticles are generated using a cyanoacrylate monomer capable of forming a polymeric matrix.

58. The method of claim 57, wherein the cyanoacrylate monomer is selected from the group consisting of butyl cyanoacrylate, isobutyl cyanoacrylate and octyl cyanoacrylate.

59. The method of claim 55, wherein the powder is characterized by a mass median aerodynamic diameter (MMAD) ranging from about 0.5 to about 10.0 μm.

60. The pharmaceutical formulation of claim 59, wherein the powder is further characterized by a geometric standard deviation (GSD) ranging from about 1.0 to about 4.0.

61. The pharmaceutical formulation of claim 53, wherein the anthracycline loaded nanoparticles have a size ranging from about 1.0 nm to about 1000 nm.

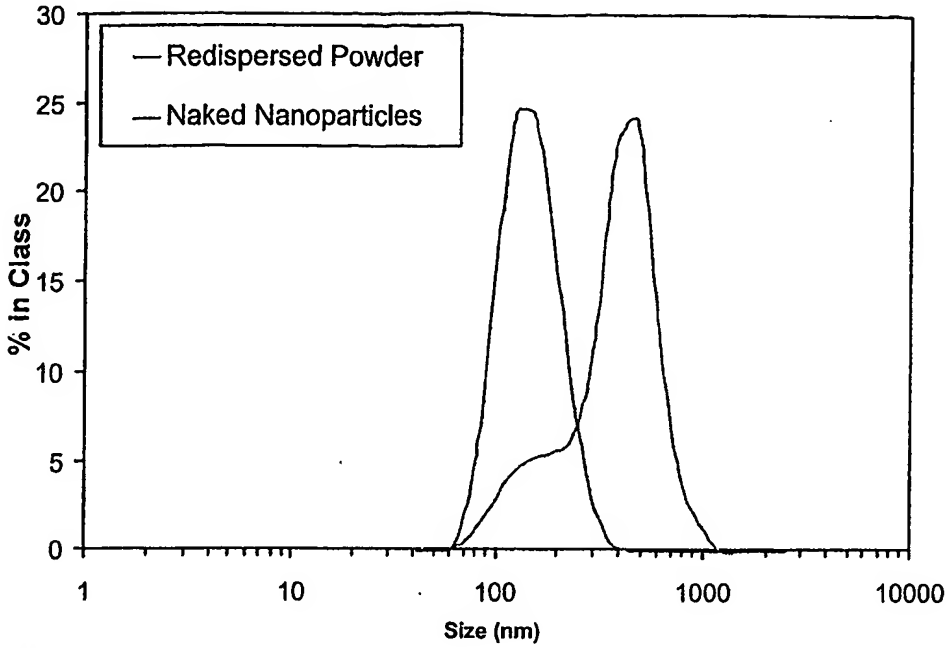


FIG. 1

H460

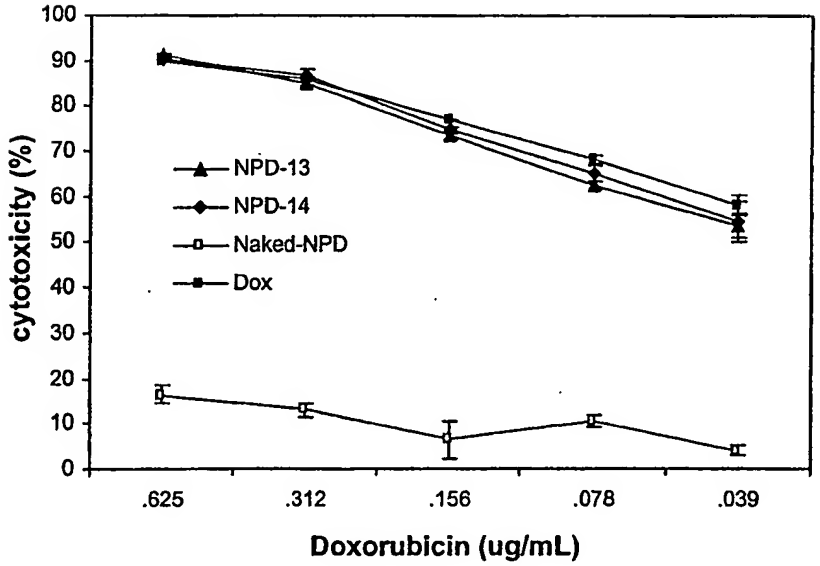


FIG. 2

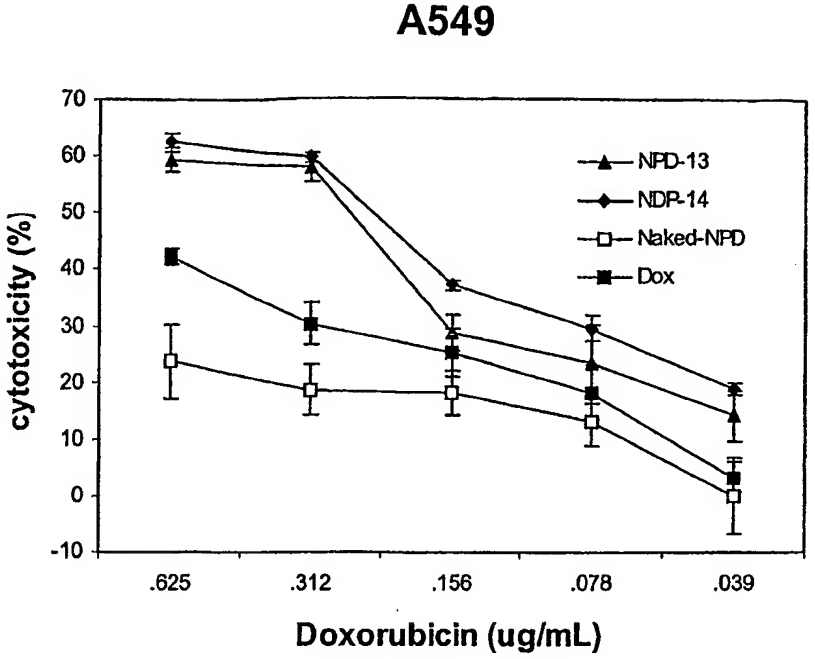
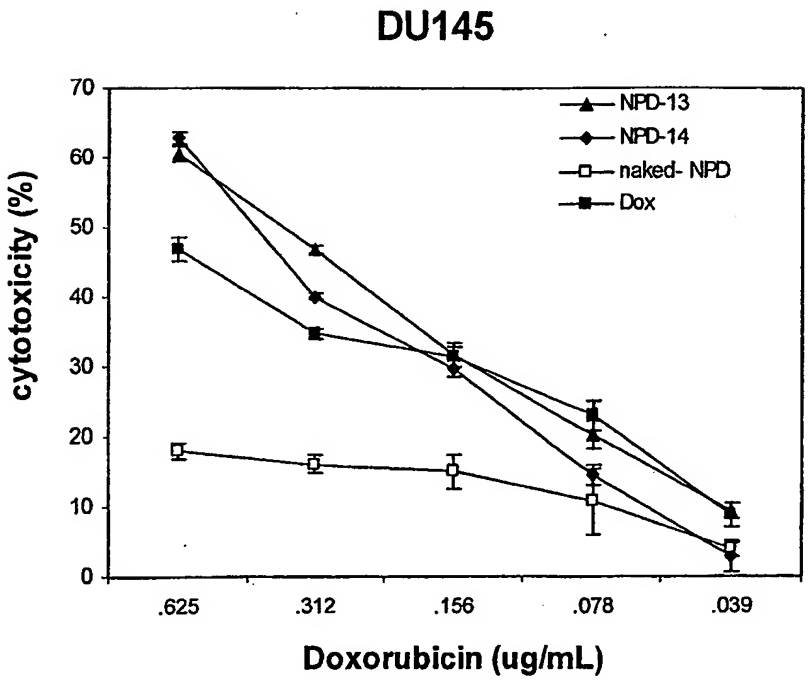

A549
70
60
50
40
30
20
10
0
-10
cytotoxicity (%)
NPD-13
NDP-14
Naked-NPD
Dox
.625
.312
.156
.078
.039
Doxorubicin (ug/mL)

FIG. 3


DU145
70
60
50
40
30
20
10
0
cytotoxicity (%)
NPD-13
NPD-14
naked- NPD
Dox
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.312
.156
.078
.039
Doxorubicin (ug/mL)

FIG. 4

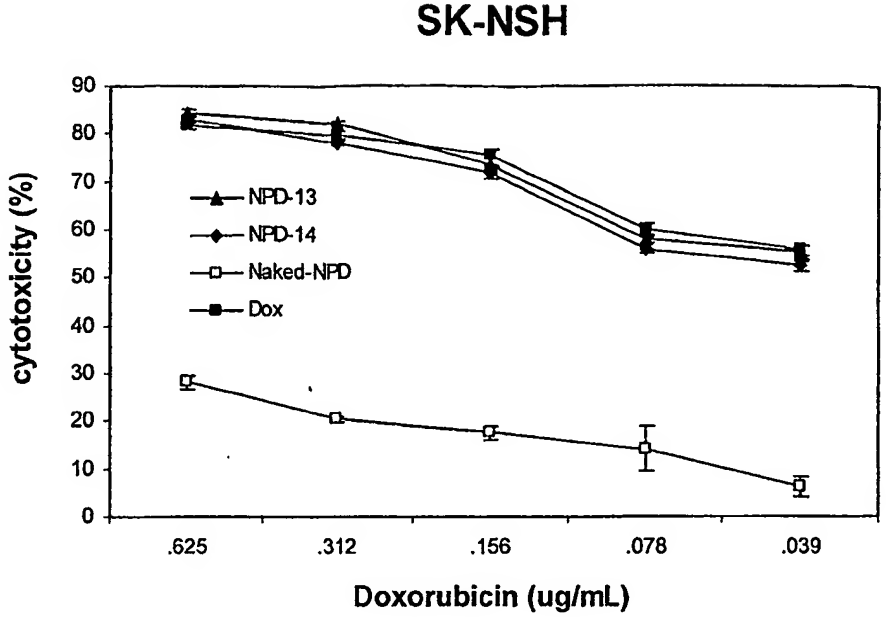
SK-NSH
cytotoxicity (%)
90
80
70
60
50
40
30
20
10
0
NPD-13
NPD-14
Naked-NPD
Dox
.625
.312
.156
.078
.039
Doxorubicin (ug/mL)

FIG. 5

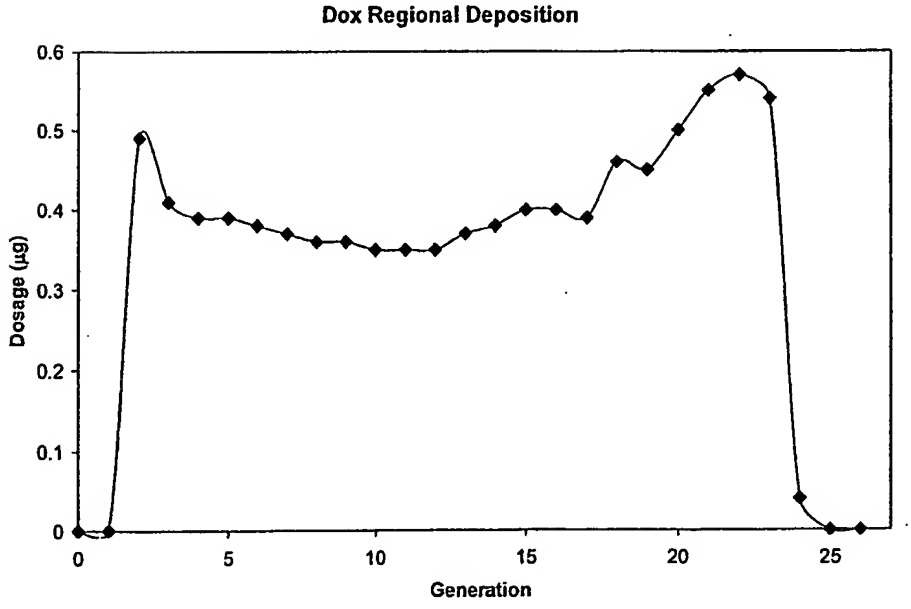
Dox Regional Deposition
Dosage (µg)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Generation

Fig. 6

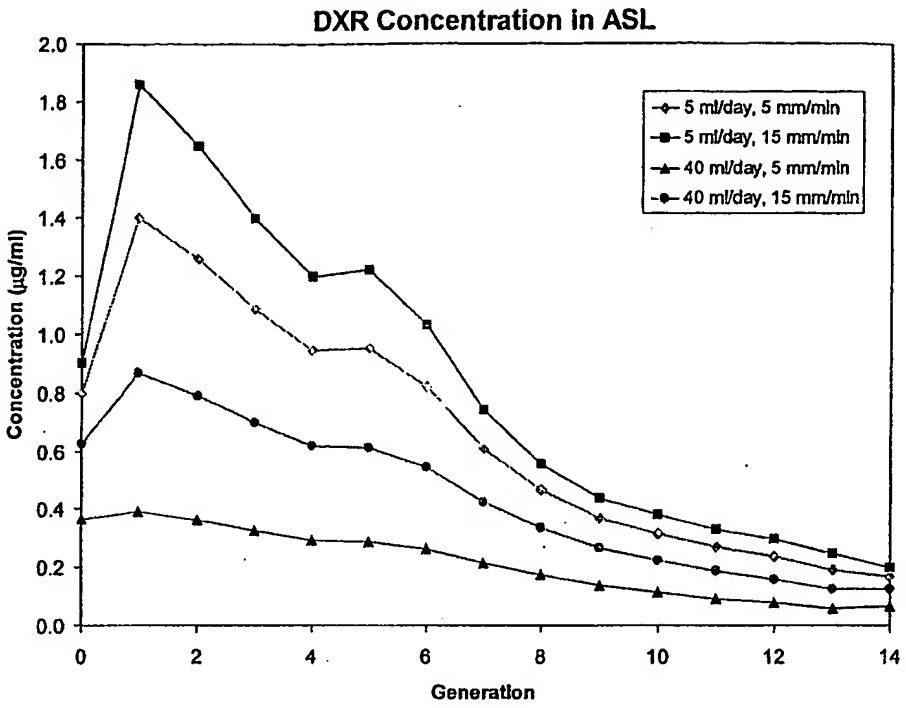
DXR Concentration in ASL
Concentration (µg/ml)
Generation
5 ml/day, 5 mm/min
5 ml/day, 15 mm/min
40 ml/day, 5 mm/min
40 ml/day, 15 mm/min
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/001306

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 9/19*** (2006.01) , ***A61P 35/00*** (2006.01) , ***A61K 47/26*** (2006.01) , ***A61K 9/72*** (2006.01) , ***A61K 9/14*** (2006.01) , ***A61K 9/12*** (2006.01) , ***A61J 3/02*** (2006.01) , ***A61K 31/704*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion, USPTO, Canadian Patent Database, Techsource

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2 435 632 (Finlay et al), 21 January, 2005 (whole document)	1-61
X	CA 2 395 887 (Chen et al), 12 July, 2001 (whole document)	1, 6, 10, 14, 17-22, 26, 30-36, 39 and 43
Y	CA 2 492 807 (Sabel et al), 04 March, 2004 (whole document)	1 - 61
Y	CA 2 488 976 (Batycky et al), 08 January, 2004 (whole document)	1-61
Y	CA 2 465 675 (Batycky et al), 30 May, 2003 (whole document)	1-61
Y	CA 2, 399, 367 (Martin et al), 16 August, 2001 (whole document)	1-61

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2006 (01-03-2006)	9 March 2006 (09-03-2006)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Authorized officer Geeta Chowdhury (819) 956-6129

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/001306

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 46-61

because they relate to subject matter not required to be searched by this Authority, namely :

The claims are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. Regardless, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims [46-61].

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/CA2005/001306

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